Colloid Polym Sci (2005) 283: 1091–1099
DOI 10.1007/s00396-004-1267-0

ORIGINAL CONTRIBUTIONS

Tao Zou
Si-Xue Cheng
Ren-Xi Zhuo

Synthesis and enzymatic degradation of end-functionalized biodegradable polyesters

Received: 13 September 2004
Accepted: 30 November 2004
Published online: 2 April 2005

T. Zou · S.-X. Cheng (✉) · R.-X. Zhuo
Key Laboratory of Biomedical Polymers of Ministry of Education,
Department of Chemistry,
Wuhan University, Wuhan, 430072,
People's Republic of China
E-mail: chengsixue@hotmail.com
Tel.: +86-27-68754509
Fax: +86-27-68754509

Abstract End-functionalization of biodegradable polymers/oligomers based on L-lactide and glycolide by cholesteryl moiety was investigated. We established the feasibility of preparing the functionalized polymers/oligomers, Chol-$(LG)_{m+n}$, through ring-opening copolymerization initiated by cholesterol bearing a hydroxyl group, without adding any catalyst. The functionalized polymers/oligomers of different molecular weights were obtained by controlling the feed ratio of the initiator cholesterol to the monomers. The chemical structure of end-functionalized polymers/oligomers was confirmed by FTIR and ^{1}H NMR. Incorporation of cholesteryl moiety into the polymer chains induces liquid crystallinity in the resultant oligomers when the molecular chains are not very long. The enzymatic degradation studies, for all the samples, were carried out using enzyme, proteinase K. Interestingly, the enzymatic degradation of cholesteryl end-functionalized polymers/oligomers resulted in a lamella-like porous structure on the sample surface, which is altogether different from the commonly reported spherical-pore structure formed during the degradation of conventional polyesters.

Keywords Biodegradable · Functionalization · Polyesters

Introduction

Biodegradable polymers belong to the class of the most attractive biomaterials because of their wide applications in biomedical fields. Aliphatic polyesters, such as polylactide (PLA), polyglycolide (PGA) and their copolymer poly(lactide-co-glycolide) (PLGA), are the most commonly used and extensively studied biodegradable polymers because of their good biocompatibility, low immunogenicity and satisfactory mechanical properties [1].

To satisfy the requirements of specific medical applications, many modification strategies have been used to optimize certain properties of the biomaterials, such as bioactivity and biocompatibility. Recently, the modification strategies involving the incorporation of some bioactive or biocompatible compounds, including lipids, vitamins, hormones, and peptides, to the polymer chains have evoked a lot of interest [2–5]. Cholesterol is a fundamental component of mammalian membranes. Stupp et al. reported that use of cholesteryl oligo (L-lactic acid) to modify PLA promoted cell adhesion [3]. In their study, cholesteryl oligo(L-lactic acid) was synthesized by the ring-opening polymerization of L-lactide initiated by aluminum alkoxide, formed by the reaction of triethyl-aluminum with cholesterol [4, 5].

Because of the wide application and importance of PLGA in biomedical fields, we investigated the end-functionalization of biodegradable polymers/oligomers based on L-lactide and glycolide by cholesteryl moiety in this study. We used cholesterol, with a hydroxyl group

as an initiator, to initiate the bulk ring-opening polymerization of L-lactide and glycolide, without adding any catalyst, which is quite different from Stupp's method. A series of polymers/oligomers, end-functionalized by cholesteryl moiety, were obtained by the one-step reaction conveniently. Cholesterol was selected in our molecular design because of its high thermodynamic affinity for the cell membrane, universally important function in all eukaryotic cells, and homeostasis for cell survival [6–8]. Because these properties are undoubtedly attractive and important for cell attachment and proliferation, these cholesteryl end-functionalized polymers/oligomers have promising applications in tissue engineering. Another consideration to incorporate cholesteryl moiety into our polymers/oligomers is based upon the mesogenic nature induced by cholesteryl moiety, which may have the potential for preparation of self-assembling drug delivery systems.

Yet another important point of this study is that the end-functionalized polymers/oligomers were obtained by the ring-opening polymerization without addition of any catalyst. This synthesis process is very useful in biopolymer field since it can be used to prepare the biodegradable polymers with good biocompatibility without adding any toxic impurity.

Experimental

Materials

Cholesterol (Bio Life Science & Technology Co. Ltd., Shanghai, China) was purified by recrystallization from ethanol. L-Lactide (Aldrich) and glycolide (Beijing Conan Polymer R & D Center) were purified by recrystallization from ethyl acetate.

Ring-opening polymerization

The polymers/oligomers end-functionalized by cholesteryl moiety were synthesized by the ring-opening polymerization of L-lactide and glycolide using cholesterol, with a hydroxyl group as an initiator, without adding any catalyst. End-functionalized polymers/oligomers with different feed ratios of the initiator cholesterol to the monomers L-lactide and glycolide were prepared. The cholesterol/lactide/glycolide feed ratios for end-functionalized polymers/oligomers coded **1**, **2**, **3**, **4** and **5** were 1/2/2, 1/5/5, 1/10/10, 1/20/20 and 1/40/40, respectively. The details of polymerization reactions are as follows. For each polymerization, a mixture of cholesterol, L-lactide and glycolide (total amount 500 mg), with a certain molar ratio, was prepared by grinding cholesterol and the monomers together. The well-mixed mixture was placed in a thoroughly dried silanized glass flask with a magnetic stirring bar. The flask was evacuated, purged with N_2 three times, sealed and then immersed in an oil bath, which was preheated to 160 °C, to carry out the polymerization. After 24-h of reaction, the product obtained was dissolved in THF and then precipitated in methanol to remove unreacted monomers. The precipitated polymer was then isolated by filtration, washed several times with ether to remove unreacted cholesterol, and finally dried under vacuum conditions for 24 h.

For comparison, PLGA was synthesized by the ring-opening polymerization under the same conditions without adding cholesterol, using $Sn(Oct)_2$ as a catalyst. The concentration of $Sn(Oct)_2$ used was 0.1 mol%.

Characterization

Fourier transform infrared spectroscopy (FTIR)

Polymers/oligomers were characterized by Fourier transform infrared spectroscopy (FTIR) (PerkinElmer-2 spectrometer). FTIR (KBr): PLGA: 1,759 cm^{-1} (C = O in lactyl and glycolyl units). All polymers/oligomers end-functionalized by cholesteryl moiety had similar spectra with both signals from cholesteryl moiety and lactyl and glycolyl units: 1,631 cm^{-1} (C = C in cholesteryl moiety), and 1,759 cm^{-1} (C = O in lactyl and glycolyl units).

Nuclear magnetic resonance (NMR)

The ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Mercury VX-300 spectrometer. The solvent for samples, cholesterol and Chol-$(LG)_{m+n}$, was $CDCl_3$, and the solvent for PLGA was DMSO-d_6.

Gel permeation chromatography (GPC)

Gel permeation chromatography (GPC) was used to determine the molecular weights of polymers. GPC analysis was performed using Waters HPLC system equipped with a 2690D separation module and a 2410 refractive index detector. Chloroform was used as an eluent and the flow rate was 1.0 ml/min.

Polarizing light microscopy (PLM)

The polymers/oligomers were observed by using polarizing light microscope (Olympus BX51) with a heating stage (Linkam THMS-600). The samples were visualized using crossed polarizers in between which a retardation plate of 530 nm was inserted. The optical images were recorded by the software Linksys 2.43.

Degradation study

Oligomer/polymer pellet samples with a thickness of 50 μm and a diameter of 5 mm were prepared by pressing the sample sandwiched between two glass slides on a heating stage (Linkam THMS-600) at 160 °C, followed by rapid cooling at room temperature. After removing one of the glass slides, the enzymatic-degradation study of the cholesteryl end-functionalized polymers/oligomers was carried out in 3 ml of phosphate buffer solution (pH = 7.4) containing 0.6 mg of proteinase K at 37 °C in a shaking water bath. The degradation medium was changed every 48 h to restore the original level of enzymatic activity. For a given time interval, samples were withdrawn from the degradation medium, and washed with distilled water. After wiping, the samples were vacuum dried at room temperature before being subjected to analysis. For control comparison, the hydrolytic degradation study was carried out under the same conditions in the absence of proteinase K. The surface morphologies of samples, before and during degradations, were observed using Hitachi X650 scanning electron microscope (SEM).

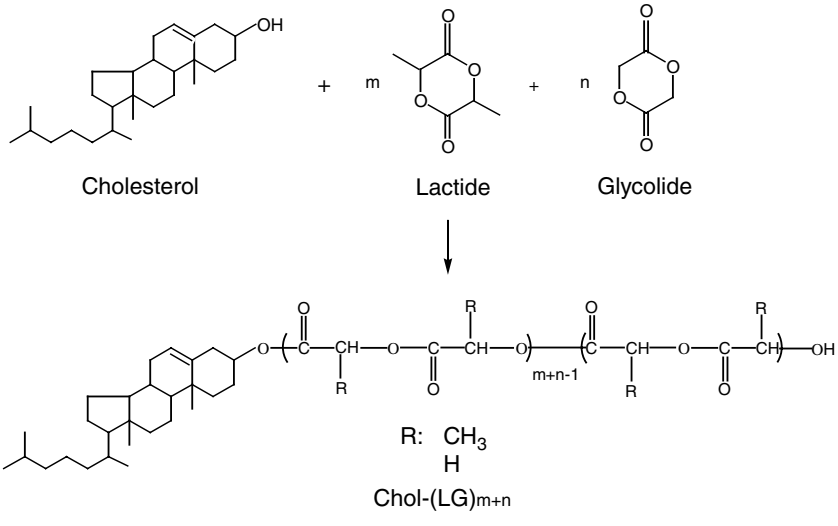
Results and discussion

Synthesis and characterization of end-functionalized polymers/oligomers

As shown in Scheme 1, Chol-$(LG)_{m+n}$ was synthesized by the ring-opening polymerization of L-lactide and glycolide without any catalyst. In our investigation, all polymers/oligomers were synthesized under rigorous anhydrous conditions. Monomers and initiator cholesterol were carefully dried to avoid the initiation by water, which would lead to a mixture of PLGA and Chol-$(LG)_{m+n}$. The optimized ring-opening polymerization conditions were identified to be 24 h at 160 °C. Under the optimized reaction conditions, the monomers, L-lactide and glycolide, can be efficiently initiated by the OH group of cholesterol thus leading to the formation of Chol-$(LG)_{m+n}$ after polymerization.

The chemical structure of functionalized polymers was verified by FTIR and ^{1}H NMR. Figure 1 shows the ^{1}H NMR spectra of cholesterol, PLGA, and Chol-$(LG)_{m+n}$ **2** as a typical example of functionalized polymers/oligomers. In the spectrum of Chol-$(LG)_{m+n}$ **2**, the typical signals from the cholesteryl moiety and the lactyl and glycolyl units can be observed at 0.68, 0.86, 0.88, 0.94 ppm (cholesteryl moiety: C**H**$_3$), 5.34 ppm (cholesteryl moiety: C**H** = C), 4.65 ppm (cholesteryl moiety: C**H**OCO), 1.60 ppm (lactyl unit: C**H**$_3$), 5.20 ppm (lactyl unit: C**H**) and 4.84 ppm (glycolyl unit: C**H**$_2$). By comparing the ^{1}H NMR spectra of cholesterol and Chol-$(LG)_{m+n}$ **2**, we find that the signal at 3.49 ppm (C**H**OH) in cholesterol disappears after the reaction because the OH group changes to OCO and the signal of the pervious **H** shifts to the lower field, indicating there is no residual cholesterol existing in the product of polymerization initiated by cholesterol. From these ^{1}H NMR spectra, we confirm that the ring-opening polymerization initiated by OH group is successful. Because of the different chemical environments caused by the different sequences in a polymer chain, the resonance of a particular H atom usually separates into several peaks. A good example is the resonance at 4.84 ppm in ^{1}H NMR spectrum of Chol-$(LG)_{m+n}$ **2**, which separates into several peaks. From this spectrum, we can conclude that the two kinds of repeating units are randomly distributed in the polymer chains [9, 10].

Scheme 1 Synthesis of Chol-$(LG)_{m+n}$



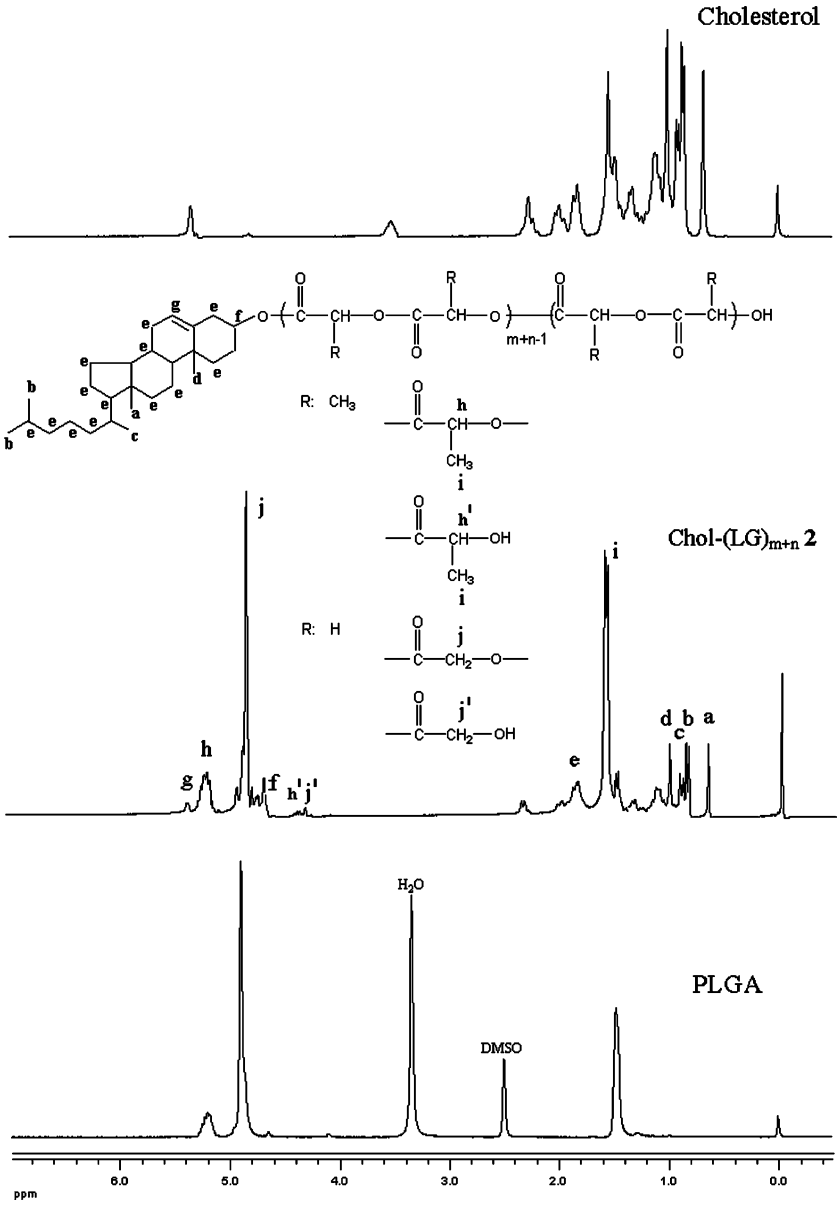


Fig. 1 ^{1}H NMR spectra of cholesterol, Chol-(LG)$_{m+n}$ **2** and PLGA

From the ^{1}H NMR spectra, we can further calculate the molar ratio of cholesterol to lactide repeating units and glycolide repeating units in the polymers/oligomers by comparing the integration values of the C**H**$_3$ peak (0.68 ppm) of cholesteryl moiety, the C**H** peak (5.20 ppm) of lactide repeating units and the C**H**$_2$ peak (4.84 ppm) of glycolide repeating units. The results are listed in Table 1. The ratios of lactide repeating units to glycolide repeating units in the copolymers are lower than the corresponding monomer feed ratios for all the samples, indicating glycolide has a higher reactivity as compared to lactide under the reaction conditions we investigated. For samples **1**, **2** and **3**, the numbers of lactide repeating units and glycolide repeating units, *m* and *n*, are higher than the monomer feed amounts, especially for the samples with high cholesteryl content. Whereas for samples **4** and **5**, *m* and *n* are lower than the monomer feed amounts. This result is quite reasonable and the explanation is as follows. If the initiator content in the reaction system is relatively high, the polymerization proceeds rapidly and the chain length increases fast once the initiation starts. As a result, a small number of cholesterol molecules may still remain in the polymerization system, which are removed by washing with ether after the reaction. In contrast, if the initiator concentration in the reaction system is very low, all cholesterol molecules become initiation centers, thus a small number of monomers may still remain in the

Table 1 Synthesis and molecular weights of Chol-$(LG)_{m+n}$ polymers/oligomers and PLGA

Polymer/ oligomer	Feed ratio cholesterol/ lactide/glycolide (mol/mol/mol)	^{1}H NMR Lactide repeating unit/glycolide repeating unit in polymer/oligomer m/n	Yield (%)	GPC	
				M_n (g/mol)	M_w/M_n
1	1/2/2	2.8/4.0	40	1,790	1.80
2	1/5/5	5.2/7.6	42	2,324	1.84
3	1/10/10	10.0/12.0	48	4,490	1.60
4	1/20/20	13.3/17.0	53	6,140	1.31
5	1/40/40	28.6/37.4	59	8,168	1.34
PLGA	0/50/50	50.0/50.3	97	26,378	1.71

polymerization system although the unreacted monomers are removed after polymerization.

Because there is no residual unreacted cholesterol existing in the products, by comparing the integration values of C**H** (h') and C$\mathbf{H}_2$ (j') of the terminal lactyl unit and terminal glycolyl unit and the integrals of the cholesteryl moiety, we can determine the degree of end functionalization, which is defined as the end-functionalized polymers/oligomers as a portion of all polymerization products. For Chol-$(LG)_{m+n}$ **1**, **2** and **3**, the signals are strong enough to perform the calculation, the calculated degrees of end functionalization for Chol-$(LG)_{m+n}$ **1**, **2** and **3** are 1.0, 0.9 and 1.0, respectively. However, for Chol-$(LG)_{m+n}$ **4** and **5**, it is very difficult to identify the signals unambiguously due to the relatively low content of the end groups.

In our study, a series of functionalized polymers/oligomers with different molecular weights were synthesized. As shown in Table 1, the molecular weight of Chol-$(LG)_{m+n}$ could be controlled by adjusting the feed ratio of the initiator cholesterol to the monomers. The molecular weight M_n increases with the decreasing initiator content, indicating that the OH groups in cholesterol molecules are effective propagation centers. GPC results show that all functionalized polymers/oligomers prepared have unimodal molecular weight distributions, and the molecular weight distributions for all samples are reasonably narrow, ranging from 1.31 to 1.84.

Because of the mesogenic nature of cholesteryl moiety, theoretically, the end-functionalized polymers/oligomers with certain molecular weights should exhibit liquid crystallinity in particular temperature ranges. In order to examine the liquid crystallinity of Chol-$(LG)_{m+n}$, we characterized our samples by PLM. The PLM images are shown in Fig. 2, and transition temperatures are summarized in Table 2. As expected, the samples **1**, **2**, and **3** show liquid crystallinity at certain temperature ranges. For example, sample **1** shows a transition of solid state to liquid crystalline state at 66 °C, and a transition of liquid crystalline state to isotropic state at 116 °C. A similar phenomena could also be observed for samples **2** and **3**. The transition temperatures increase with the increasing molecular weight. The typical liquid crystal textures observed in the temperature ranges in which liquid crystallinity

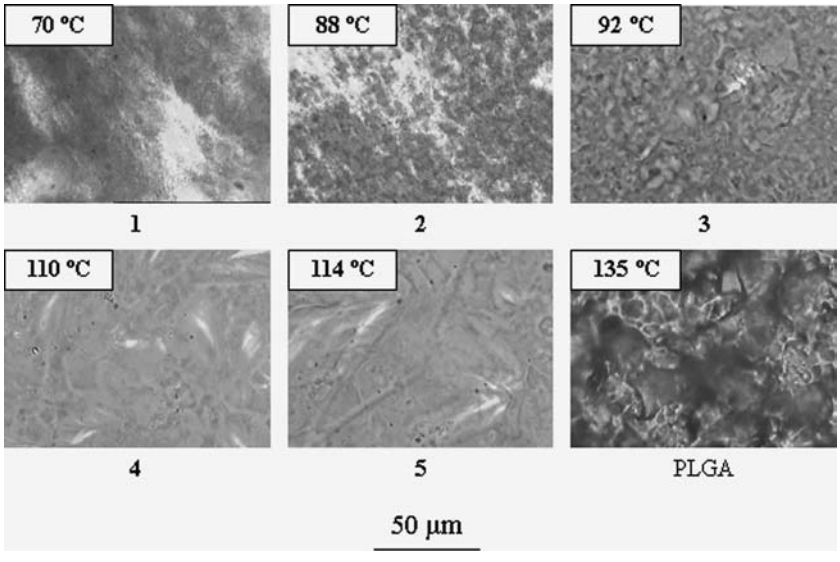


Fig. 2 PLM images of Chol-$(LG)_{m+n}$ **1**, **2**, **3**, **4**, **5** and PLGA

Table 2 Properties of Chol-$(LG)_{m+n}$ polymers/oligomers

Polymer/oligomer	PLM Temperature range in which liquid crystallinity is exhibited (°C)
1	66–116
2	80–125
3	86–128
4	No obvious liquid crystallinity
5	No obvious liquid crystallinity

exhibits are shown in Fig. 2 (**1**, **2**, and **3**). For samples **4** and **5**, no clear liquid crystallinity could be observed because of the low content of cholesteryl moiety in the polymer chain. The morphologies during the melting of samples **4**, **5** and PLGA are also shown in Fig. 2.

Degradation of end-functionalized polymers/oligomers

The enzymatic degradation of PLA polymers has been investigated by several groups [11–14]. Proteinase K is one of the enzymes having significant effect on the degradation of poly(L-lactide) [11]. It was reported that proteinase K preferentially degraded L-lactyl units as opposed to D-lactyl units [12–14]. In our study, both enzymatic degradation and hydrolytic degradation of all the end-functionlized polymers/oligomers and PLGA were investigated. The surface morphologies of Chol-$(LG)_{m+n}$ **1**, **2**, **3**, **4**, **5** and PLGA before degradation and after degradation for 24 h are compared in Fig. 3, and the corresponding weight losses of these samples are listed in Table 3. Before degradation, all samples had smooth surface (Fig. 3a). After enzymatic degradation for 24 h (Fig. 3b), Chol-$(LG)_{m+n}$ **1** showed a porous surface and with the weight loss of 14.5 wt%. A few pores appeared on the surfaces of Chol-$(LG)_{m+n}$ **2** and **3**. The surfaces of Chol-$(LG)_{m+n}$ **4** and **5** were highly porous. Consistently, these two samples had very high weight loss values, 85.0 wt% and 80.0 wt%, respectively. The enzymatic degradation also resulted in the formation of a lot of pores on the surface of PLGA. Clearly, proteinase K showed significant effect on the degradation for all the samples. The difference in the weight loss values for different samples indicate there are two factors affecting the degradation rate: the molecular weight of the samples and the content of cholesteryl moiety in the polymers/oligomers. Taking account of the molecular weight, according to the previous studies [15], the lower molecular weight results in faster degradation rate. In view of cholesteryl moiety content, our experimental results strongly imply that a higher cholesteryl moiety content causes a slower degradation rate. In our investigation, the sample with a higher cholesteryl moiety content had a lower molecular weight. As a overall effect of these two competitive factors, Chol-$(LG)_{m+n}$ **4** and **5** had relatively fast degradation rates because their low cholesteryl moiety content had a dominant effect.

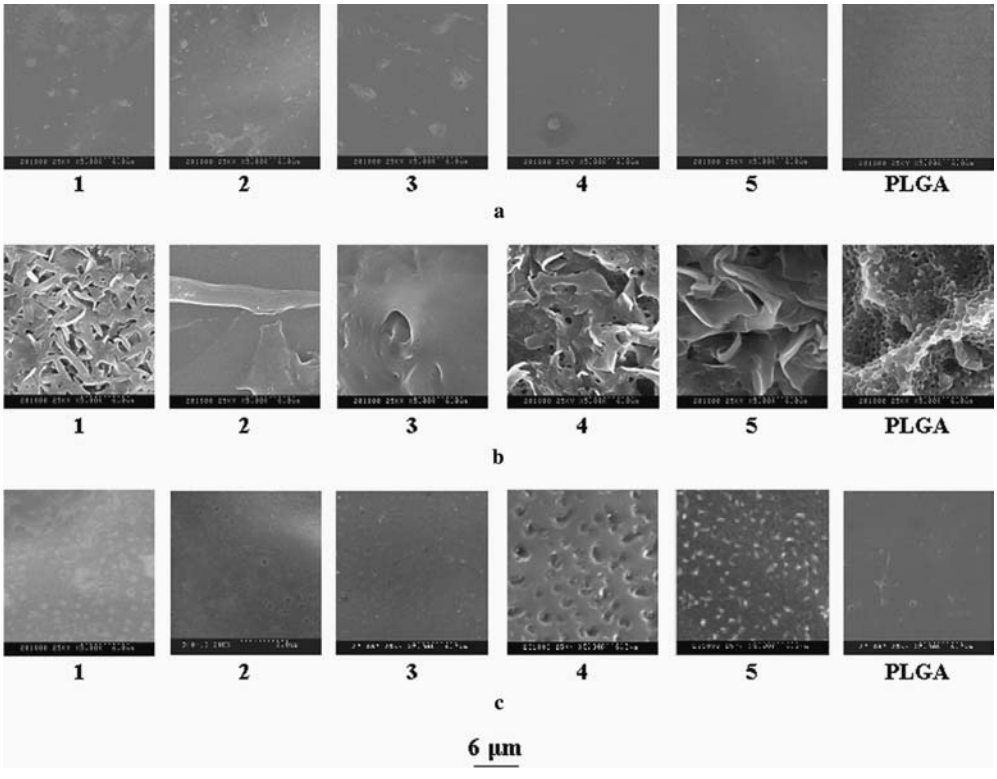


Fig. 3 SEM images of Chol-$(LG)_{m+n}$ **1**, **2**, **3**, **4**, **5** and PLGA, **a** before degradation, **b** after enzymatic degradation for 24 h, **c** after hydrolytic degradation for 24 h

Table 3 Weight losses of Chol-$(LG)_{m+n}$ polymers/oligomers and PLGA after degradations for 24 h

Polymer/ oligomer	Weight loss after enzymatic degradation for 24 h (wt%)	Weight loss after hydrolytic degradation for 24 h (wt%)
1	14.5	2.0
2	5.9	4.5
3	16.4	5.4
4	85.0	14.2
5	80.0	6.8
PLGA	62.7	0

Chol-$(LG)_{m+n}$ **2** and **3** had relatively slow degradation rates as compared to Chol-$(LG)_{m+n}$ **4** and **5** since their high cholesteryl moiety content had a critical role to play. For comparison, the morphologies of the samples after 24-h hydrolytic degradation in the absence of enzyme are shown in Fig. 3c. Chol-$(LG)_{m+n}$ **4**, which had the highest weight loss, showed a porous surface. For Chol-$(LG)_{m+n}$ **1**, **2**, **3** and **5**, only small pores could be observed on the sample surfaces. For PLGA, no obvious morphological change and weight loss was detected after 24-h hydrolytic degradation.

Figure 4a1 shows the surface morphologies of Chol-$(LG)_{m+n}$ **2** after enzymatic degradation for different time intervals, and Fig. 4a2 shows the surface mor-

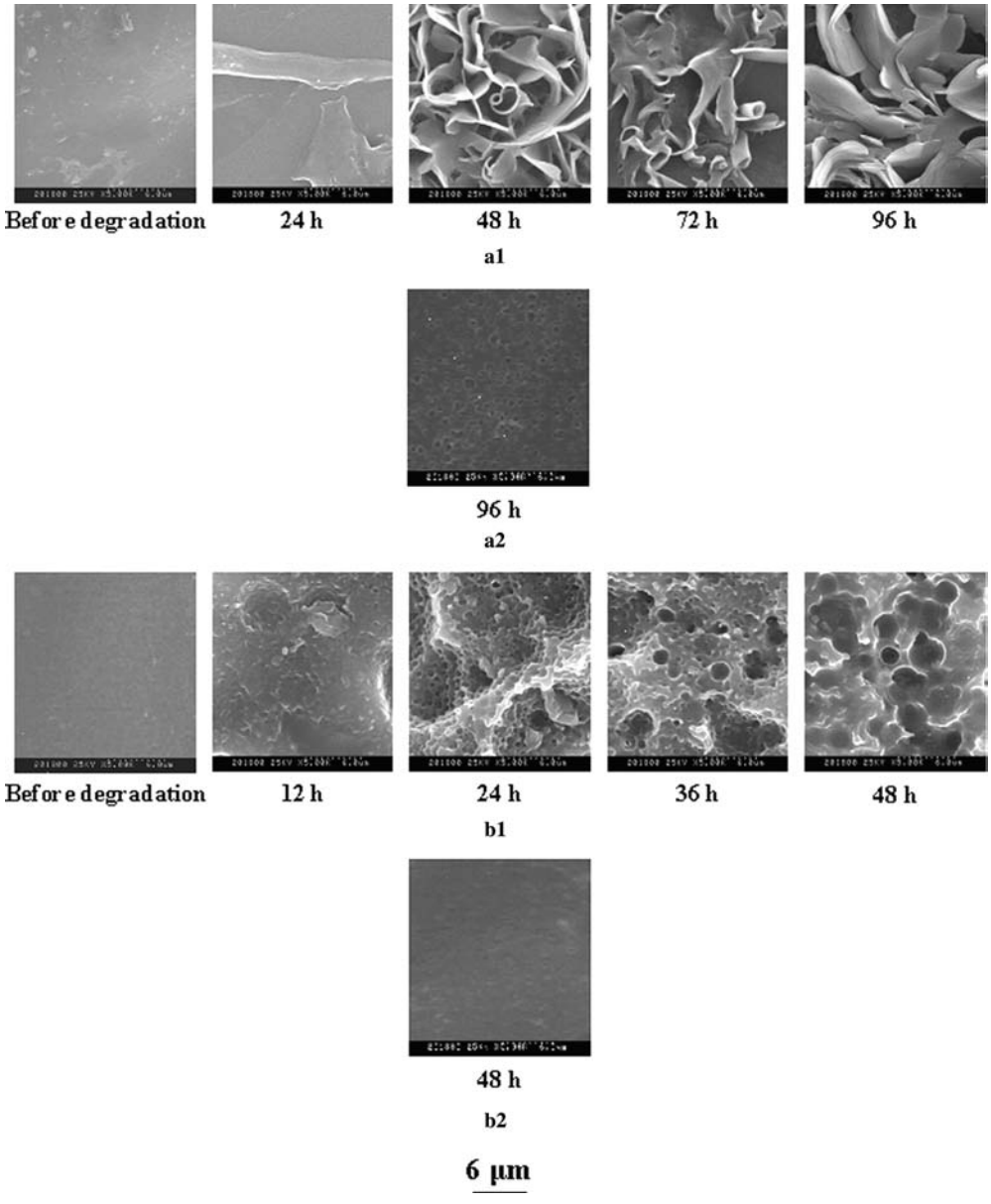


Fig. 4 SEM images of Chol-$(LG)_{m+n}$ **2** and PLGA, **a1** Chol-$(LG)_{m+n}$ **2** after enzymatic degradation for different time intervals, **a2** Chol-$(LG)_{m+n}$ **2** after hydrolytic degradation for 96 h, **b1** PLGA after enzymatic degradation for different time intervals, **b2** PLGA after hydrolytic degradation for 48 h

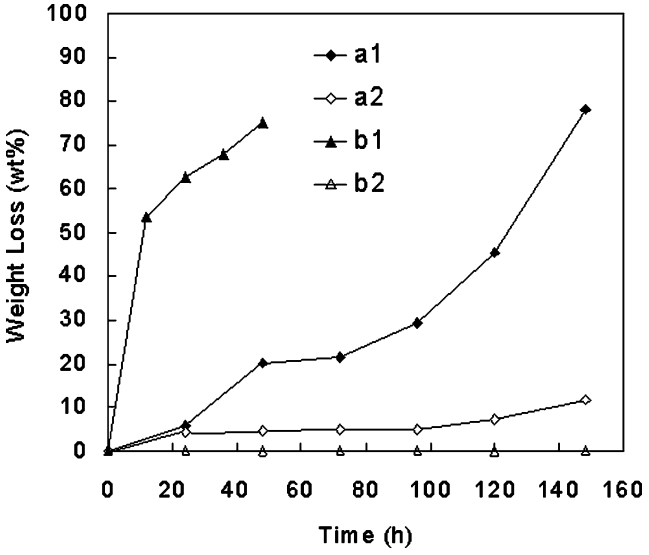


Fig. 5 Comparison of weight losses during degradations under different conditions: **a1** enzymatic degradation of Chol-$(LG)_{m+n}$ **2** in the presence of proteinase K, **a2** hydrolytic degradation of Chol-$(LG)_{m+n}$ **2** in the absence of proteinase K, **b1** enzymatic degradation of PLGA in the presence of proteinase K, and **b2** hydrolytic degradation of PLGA in the absence of proteinase K

phology of Chol-$(LG)_{m+n}$ **2** after hydrolytic degradation for 96 h. The surface morphologies of PLGA after enzymatic degradation and hydrolytic degradation are presented in Fig. 4b1, b2, respectively. After the enzymatic degradation, a lot of pores formed on the surface of PLGA (Fig. 4b1). The pores were spherical in shape with circular interconnections. This kind of connected spherical-pore structure has been widely reported in the previous literatures [13, 14, 16]. Interestingly, the enzymatic degradation of Chol-$(LG)_{m+n}$ **2** resulted in a totally different surface morphology (Fig. 4a1)—the lamella-like porous structure. Based on our observation, we found that all the cholesteryl end-functionalized polymers/oligomers showed a similar lamella-like porous structure when their weight loss values reached a certain level during enzymatic degradations. This interesting phenomenon might be because of the existence of cholesteryl moiety in the polymer/oligomer chains, which resulted in the different degradation rates in different regions of the sample surfaces. During hydrolytic degradation, only spherical pores could be observed for Chol-$(LG)_{m+n}$ **2** for the same degradation time. The weight losses of Chol-$(LG)_{m+n}$ **2** and PLGA after different degradation time intervals are shown in Fig. 5. During the enzymatic degradation, Chol-$(LG)_{m+n}$ **2** had a much lower weight loss as compared to PLGA for the same time intervals. Whereas, during the hydrolytic degradation, the weight losses of Chol-$(LG)_{m+n}$ **2** were higher than that of PLGA. As mentioned earlier, during the enzymatic degradation, the high cholesteryl moiety content in Chol-$(LG)_{m+n}$ **2** had a dominant effect, which resulted in the slow degradation rate. While, during the hydrolytic degradation, the low molecular weight of Chol-$(LG)_{m+n}$ **2** played an important role, leading to a higher weight loss value. For PLGA with a relatively high molecular weight, in the initial hydrolytic degradation stage, no obvious weight loss could be detected even when the degradation might have resulted in a decrease in the molecular weight.

Conclusions

Novel biodegradable polyesters end-functionalized by cholesteryl moieties were successfully synthesized by the ring-opening copolymerization of L-lactide and glycolide initiated by cholesterol, bearing a hydroxyl group, without adding any catalyst. The polymers/oligomers with different molecular weights could be obtained by adjusting the initiator/monomer feed ratio. Because of the incorporation of cholesteryl moiety, some oligomers exhibited liquid crystallinity in particular temperature ranges. Proteinase K shows a significant effect on the degradation for all samples. Two competitive factors affect the degradation rate: the molecular weight of the samples and the content of cholesteryl moiety in the polymers/oligomers. Interestingly, the enzymatic degradation of cholesteryl end-functionalized polymers/oligomers results in the lamella-like porous structure on the sample surfaces, which is totally different from the connected spherical- pore structure formed during degradation of PLGA.

Acknowledgments This research was supported by grant (20204010) from the National Natural Science Foundation of China. One of the authors, Si-Xue Cheng, is grateful to the Ministry of Education of China for the financial support under "Trans-Century Training Programme Foundation for the Talents" and Wuhan University for the grant of "Innovative Project Foundation for Young Scientists". Special thanks are due to Ms Qing-Rong Wang for the GPC measurement.

References

1. Ignatius AA, Claes LE (1996) Biomaterials 17:831
2. Kricheldorf HR, Kreiser-Saunders I (1994) Polymer 35:4175
3. Hwang JJ, Iyer SN, Li LS, Claussen R, Harrington DA, Stupp SI (2003) PNAS 99:9662
4. Klok HA, Hwang JJ, Stupp SI (2002) Macromolecules 35:746
5. Klok HA, Hwang JJ, Hartgerink JD, Stupp SI (2002) Macromolecules 35:6101
6. Simons K, Ikonen E (2000) Science 290:1721
7. Urata K, Takaishi N (2001) Eur J Lipid Sci Technol 103:29
8. Koyama Y, Ito T, Kimura T, Murakami A, Yamaoka T (2001) J Control Rel 77:357
9. Kasperczyk J (1996) Polymer 37:201
10. Breitenbach A, Pistel KF, Kissel T (2000) Polymer 41:4781
11. Williams DF (1981) Eng Med 10:5
12. MacDonald RT, McCarthy SP, Gross RA (1996) Macromolecules 29:7356
13. Li S, Girard A, Garreau H, Vert M (2001) Polym Degrad Stabil 71:61
14. Li S, Tenon M, Garreau H, Braud C, Vert M (2000) Polym Degrad Stabil 67:85
15. McColluch I, Shalaby S (eds) (1998) Tailored polymeric materials for controlled delivery systems. ACS symposium series 709, American Chemical Society, Washington DC, p 242
16. Cai Q, Shi G, Bei J, Wang S (2003) Biomaterials 24:629